Ernest E. McConnell, D.V.M., M.S. (Path), DACVP, DABT
President, ToxPath, Inc.

Office Telephone/FAX
919-848-1576
toxpathmcc@bellsouth.net

3028 Ethan Lane
Laurdane Est.
Raleigh, NC 27613

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Review of the Draft NTP SAN Trimer Technical Report (TR-573)

Prepared by Dr. Ernest E. McConnell at the Request of the SAN Trimer Association
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I would like to take this opportunity to comment on specific pathology issues relating to the draft Technical Report on San Trimer. I will restrict my comments to the finding of spinal cord nerve degeneration in male rats as to whether it is treatment related or not. To do this I have to make a few assumptions because the exact nature of the "expanded review" of the spinal cords is not detailed enough for me to be certain what was entailed in the expanded review.

Background: I am concerned about the biological significance of spinal cord nerve degeneration in male rats as reported in the draft Technical Report. My reasons for this are as follows:

1. The expanded review of the spinal cords showed an incidence of nerve fiber degeneration in the spinal cord of 34/47 in the controls, 37/48 in the low-dose, 37/50 in the mid-dose and 43/50 in the high-dose. I think it is problematic to attach a treatment-related significance to any non-neoplastic lesion when the incidence in the treated animals is not much different from the controls, unless extreme care was taken in the approach to the expanded review (see below). Also, I find it hard to substantiate biological significance to such a small difference when considering the subtle nature (severity) of the lesion, as noted below.

2. Another factor that needs to be considered is the significant increase in the longevity of the exposed animals. It is apparent that this is a normal aging lesion because it was found in 34/47 control rats. Therefore, it is reasonable to expect it to be of higher incidence in the high-dose rats because they lived longer.

3. It is not apparent from the draft Technical Report how the amount (number) of spinal cord nerve roots was recorded. As I understand it, three levels of spinal cord (cervical, thoracic and lumbar) were "cut-in" and sections made of each level. If that is the case, there are four spinal nerves potentially available on each section, i.e., left dorsal, left

ventral, right dorsal and right ventral, for a total of 12 nerves per animal. However, in my experience one rarely finds all four on one section. Therefore, it is my assumption that the number of nerves available for examination varied from level to level and from animal to animal. So, how was the incidence recorded? For example, let's assume that 12 nerves were examined in one rat with 6 nerves showing no lesion, 3 with minimal and 3 with mild degeneration. I assume this rat would be recorded to show the lesion. But, let's further assume that only one or two nerves were available in the next rat and no nerve degeneration was observed. I assume that this rat would be recorded as not showing the lesion. However, unless nerve degeneration is an "all-or-none" phenomenon, which is unlikely for such a subtle lesion (see below), there is an obvious bias favoring finding the lesion in the first rat and not the second because of more nerves available for examination.

4. In terms of severity, it appears that the "scoring" system was that typically used by the NTP, i.e., 0 = no lesion, 1 = minimal, 2 = mild, 3 = moderate and 4 = marked/severe severity. According to the draft Technical Report, the average severity of the nerve degeneration in the spinal cord was reported to be 1.0 in the controls, 1.1 in the low-dose, 1.2 in the mid-dose and 1.3 in the high-dose. In other words, there was only a marginal difference in the severity between the control and high-dose and all control and treated groups were considered minimal. Using the example in point #2 of the rat with 12 spinal nerves, where minimal (grade 1.0) nerve degeneration was found in three nerves and mild (grade 2.0) in another three, how was the grade scored? Was it a 2.0 because this was the most severe lesion noted? Or, 1.5 because this was the average of the rats with the lesion? Or, 0.75 because it's the average of the full 12 nerves?

5. Again, in terms of severity, in my experience one typically requires a difference of a whole unit, e.g., difference of 1.0 to 2.0 or more, to be biologically significant. This is especially the case when one is working with only 50 animals/exposure group.

6. Finally, in terms of severity, this being a subtle lesion, e.g., grade-1, and part of the normal aging process in rats, the only valid way of determining a treatment-related effect would be for the reviewing pathologist to examine all of the slides from all of the animals in a fully blind fashion, e.g., with no knowledge of dose group or prior diagnosis. Was this the case?

In summary, based on the information in the draft Technical Report on San Trimer, I find no supportable evidence of a treatment-related effect on spinal cord nerve degeneration based on the pathology data. And importantly, if nerve degeneration was used to support the conclusion that San Trimer shows equivocal evidence of carcinogenic activity, I find no scientific basis for this conclusion.

Thank you for allowing me to comment on this issue.

With regards,

Ernest E. McConnell